REMARKS

The Applicants appreciate the Examiner's thorough examination of the subject application. Applicants request reconsideration of the subject application based on the following remarks.

Claims 1-7 are currently pending in the application. Claims 1-7 have been amended. Support for the amendments can be found throughout the specification as filed. Support for the amendments may be found throughout the specification. For example, support for the amendments to claims 2 and 4 may be found on page 4, line 5 from the bottom.

The Office Action takes the position that "the requirement to select a single amino acid sequence of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 21, or 23 is not to be construed as a requirement for an election of species since each of the amino acid sequences recited in alternative form is not a member of a single structurally and functionally related genus."

Applicants respectfully disagree. The polypeptides having amino acid sequences of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 17, 19, 21, and 23 are members of a single genus linked in the common concept of "enzyme exhibiting nicotianamine synthase activity." As disclosed by the specification as filed, nicotianamine synthase activity was detected in barley nicotianamine synthase (SEQ ID NO: 1, page 12, lines 1-10), rice nicotianamine synthase (SEQ ID NO: 17, page 14, lines 4-15) and Arabidopsis thaliana nicotianamine synthase (SEQ ID NO: 10, 21, and 23, page 15, line 9-18).

Moreover, the polypeptides disclosed by the present invention, e.g., SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 17, 19, 21, and 23, have high identity an d homology in their amino acid sequences as shown in page 11, table 1 of the present specification. The sequence identity and

homology of the amino acid sequences of the invention are further illustrated by the attached amino acid sequence alignment. All of the amino acid sequences of SEQ ID NOS: 3, 5, 7, 9, 11, 13, and 15 (barley nicotianamine synthase) have more than 60 % identity with the amino acid sequence of SEQ ID NO: 1. The amino acid sequence of SEQ ID NO: 17 (rice nicotianamine synthase) has 70% identity with the amino acid sequence of SEQ ID NO: 1. Each of the amino acid sequences of SEQ ID NO: 19, 21, and 23 have more than 40% identity with the amino acid sequence of SEQ ID NO: 1. Thus all of the nicotianamine synthase amino acid sequences disclosed by the present invention, e.g., SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 have the consensus sequence of $_{207}$DVVFLAALVGM$_{217}$.

Claims 1-7 were rejected under 35 U.S.C. §101 as being allegedly directed toward non-statutory subject matter.

Claim 1, as amended, provides isolated or purified enzymes having nicotianamine synthase activity. Thus, claims 1-7 are directed to statutory subject matter.

Claims 1-7 were rejected under 35 U.S.C. §112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Claims 1-7, as amended, are fully compliant with the requirements of 35 U.S.C. §112, second paragraph, including the requirement to particularly point out and distinctly claim the subject matter of the invention.

Claims 1, 2, 4, and 6 were rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to

reasonable convey to on skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Claim 1, as amended, provides an isolated or purified enzyme exhibiting nicotianamine synthase activity where the enzyme is SEQ ID NO 1 or a polypeptide differing from SEQ ID NO 1 by insertion or deletion of at least one amino acid or replacement of an amino acid with one another amino acid residue. The specification provides sufficient instruction for one skilled in the art to isolate or purify peptides with homologous amino acid sequences to SEQ ID NO: 1. Moreover, one skilled in the art can measure nicotianamine synthase activity for such peptides or enzymes based on the description provided in the specification. See, Example 2 entitled "assay of nicotianamine synthase activity."

Claims 1-7, as amended, are fully compliant with the requirements of 35 U.S.C. §112, including the requirements of §112, first and second paragraph. Thus the §112 rejections should be withdrawn.

A brief discussion of the present invention may be of assistance in understanding the differences between the claimed invention and the disclosure of Higuchi.

The present invention is based on the first successful purification of nicotianamine synthase and cloning of the gene encoding nicotianamine synthase. As is well known in the art, purification of nicotianamine synthase has proven to be very difficult in part because nicotianamine synthase is susceptible to degradation by proteases and become multiple isozomes of the enzyme exist.

Claims 1, 2, 4, and 6 were rejected under 35 U.S.C. §102(b) as being allegedly anticipated by Higuchi (1994) (Plant Soil, 165: 173-179 (1994)). The rejection is traversed.

The Office Action alleges that Higuchi (1994) teaches an isolated and purified nicotianamine synthase from barley roots and inherently comprises the amino acid sequence of SEQ ID NO: 1.

The abstract of Higuchi teaches that nicotianamine synthase was purified as one band on SDS-PAGE in a purification technique using hydrophobic chromatography, hydroxylapatite chromatography, and preparative SDS-PAGE in accordinace with the purification method described in the Materials and Methods portion of the document (see, page 174, right column lines 11-last line).

Reference AN {Higuchi (1999)} of the IDS filed July 26, 2001 with the application packet was was a follow up publication supporting the initial Higuchi (1994) disclosure. Higuchi (1999) recited the preparation of polyclonal antibodies of nicotianamine synthase and Western blot analysis to the protein. The Higuchi (1999) document further recited that nicotianamine synthase was purified by one band (see Fig 1 on page 684 which is a Western blot analysis of the total protein extracted from Fe-deficient barley roots). In a two dimensional SDS-PAGE experiment, it was demonstrated that the single band of nicotianamine synthase contained a plurality of materials (see FIG 2 on page 685, Fe-deficient root). Thus the single SDS-PAGE band disclosed in Higuichi (1994) is a crude mixture of at least two polypeptides which may include e several isozymes of nicotianamine synthase and/or additional proteins or combinations thereof.

As the reference is understood, Higuchi (1994) fails to teach or suggest any method of preparing or isolating purified nicotianamine synthase. The single SDS-PAGE band disclosed by Higuchi does not constitute a purified nicotianamine synthase protein and the inventors were not able to determine the amino acid sequence of the protein from the band.

Enclosed is a declaration by one of the named inventors, Dr. Kyoko Higuchi, which states that the polypeptide and nucleic acid of the invention could not be isolated using the purification procedure of Higuchi (1994) or any of the conventional purification processes known in the art at the time the invention was made.

Applicants have surprisingly discovered an improved purification procedure for the isolation of enzymes exhibiting nicotianamine synthase activity which procedure is disclosed at pages 20-22 of the instant specification.

The enclosed Table Comparing Purification Methods provides a comparison of the purification procedure disclosed in Higuchi (1994) and the purification procedure of the instant invention is provided for the Examiners convenience. As illustrated by the enclosed Table, the method provided by the present invention of preparing purified or isolated enzyme exhibiting nicotianamine synthase activity is substantially more complex than the procedure recited by Higuchi (1994).

In contrast, Higuchi (1994) fails to disclose isolation or purification of any enzyme exhibiting nicotianamine synthase activity. As shown in the supporting document (Higuchi (1999)), the composition isolated by Higuchi (1994) comprises a mixture of several different materials as shown by the two dimensional SDS-PAGE experiment.

Thus, for at least the reasons discussed herein, Higuchi (1994) fails to teach or suggest the purified or isolated enzymes exhibiting nicotianamine synthase activity which are provided by the present invention. Thus claim 1 is patentable over Higuchi. Claims 2-7 depend from claim 1 and are therefore also patentable over Higuchi.

Early consideration and allowance of the application are earnestly solicited.

Respectfully submitted,

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